FACTORS PRODUCING HIGH YEAST YIELDS IN SYNTHETIC MEDIA[1]

B. H. OLSON AND MARVIN J. JOHNSON

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wisconsin

Received for publication November 18, 1948

Although the nutritional factors essential for yeast growth have been extensively studied, the yeast cell yields obtained on media of known composition have always been much lower than those attainable on a natural medium. In the present investigation yeast nutritional requirements have been studied under aeration conditions shown to be optimal. A synthetic medium has been found on which yields equal to those attainable on natural media can be obtained.

A number of papers on the nutritional requirements of yeast have appeared since the review of Williams (1941). Leonian and Lilly (1942), Burkholder (1943), Burkholder, McVeigh, and Moyer (1944), and Thorne (1945) have presented data on the vitamin and nitrogen requirements of yeast. Joslyn (1941) has reviewed yeast mineral metabolism. De Becze and Liebmann (1944) and Singh, Agarwal, and Peterson (1948) have discussed the effects of aeration on yeast yields.

METHODS

Fermentation methods. The strains of yeast used in this work were obtained from Professor McCoy of the Agricultural Bacteriology Department of the University of Wisconsin. *Saccharomyces cerevisiae* y-30, which has been used for the major part of this work, is a single cell isolate from a commercial bakers' yeast.

The 500-ml Erlenmeyer flasks that were used for studying yeast growth were plugged with cotton and sterilized. Twenty-five ml of the fermentation medium, previously sterilized at 120 C for 30 minutes, were transferred aseptically to each sterile flask just before inoculation. The inoculum was prepared by making two consecutive 18-hour transfers in basal medium. In the case of the mineral studies, the element to be tested for was removed from the basal medium. If the experiment warranted, as in the study of growth factor requirements, the yeast was washed once and then resuspended in sterile distilled water before use as inoculum. Five-hundredths ml of the second transfer were used to inoculate each experimental flask. This volume contained approximately 0.15 mg of dry yeast except when the growth of the inoculum was decreased because of the absence of some metal.

After inoculation the flasks were shaken for 24 hours at 30 C on a Gump rotary shaker that described a 2¼-inch diameter circle at 253 rpm. In mineral re-

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This investigation was supported in part by a grant from Standard Brands, Inc., New York, New York.

quirement experiments, however, the fermentation time was 36 to 40 hours because of the small inoculum used. After incubation the dry cell weight in 10 ml of medium was determined. Corrections for volume changes during fermentation were made.

When available, reagent grade chemicals were used in all media. The media used in mineral studies contained only 1 gram of asparagine per liter. No citrate was added to the medium. After several procedures were tried, with little or no success, for use in removing the elements zinc, iron, and copper, the following methods were evolved: The removal of zinc from the medium was accomplished by adding 75 ml of approximately 0.1 per cent dithizone in carbon tetrachloride to each of several 400-ml portions of the complete medium at pH 5.0. The medium plus the dithizone solution was placed in 2-liter flasks and shaken overnight on a reciprocating shaker at 90 strokes per minute. The excess dithizone and the dithizone-metal complex were then removed by repeated extraction with approximately 0.1 volume of redistilled carbon tetrachloride. To ensure complete removal of the excess dithizone, three extractions were made after all visible color was removed. The medium was then sterilized and was ready for use.

Iron was removed from the medium by adding 75 ml of approximately 0.1 per cent 8-hydroxyquinoline in chloroform to each of several 400-ml portions of basal medium at pH 5.0 in 2-liter flasks. These were shaken overnight on the reciprocating shaker as for the removal of zinc. The metal complex and excess 8-hydroxyquinoline were removed by repeated extraction with 0.1 volume of redistilled chloroform. Because of the less intense color of the metal complex, five extractions were made after the disappearance of visible color. The purified medium was sterilized, and was then ready for use.

Copper was removed from the medium by the same procedure used to remove zinc except that the dithizone treatment was conducted at pH 1.5. After the removal of excess dithizone, the pH was adjusted to 5.0 with redistilled aqueous ammonia.

All mineral solutions added to the purified media were prepared with reagent grade chemicals and redistilled water. The minerals that were required in large amounts were not removed from the basal media, since the amount present in the original media was negligible in comparison with the amounts added. All 500-ml flasks used for yeast growth in the mineral studies were specially cleaned. Each flask was washed in dichromate cleaning solution, then rinsed ten times in distilled water, rinsed three times in redistilled water, and dried in an inverted position.

It has not been reported that yeast has an optimum aeration level. It must always be assumed that aeration limits the yield unless it can be shown that more effective aeration does not increase yields. To provide a means of determining when aeration ceases to be limiting, a fermenter capable of giving extremely high levels of aeration was designed. This fermenter was made of stainless steel. It was 6 inches in diameter and had a total volume of 3.5 liters. It was provided with a variable speed agitator with a speed range of 800 to 1,600 rpm. The impeller on the agitator shaft was 4 inches in diameter with 8 blades, each bent to a 30° pitch, arranged for upward thrust. The air was introduced through a cotton filter to the sparger located under the impeller. The sparger directed the air up into the impeller through $\frac{1}{32}$-inch holes. The 13 holes were spaced $\frac{3}{4}$ inch apart. A baffle plate 1 inch wide was used to prevent coning.

Analytical methods. All sugar determinations were made with the Shaffer-Somogyi (1933) reagent 50 with 5 g potassium iodide. Iron determinations were made with the α,α'-dipyridyl method of Jackson (1938). Zinc and copper were determined by the dithizone mixed color methods given by Sandell (1944).

A rapid procedure was developed from the method of Sandell (1944) for the wet ashing of samples for metal analyses. An amount of medium (usually more than a liter), containing enough of the desired element to be in the range of the analytical method, was evaporated to a heavy syrup. To the syrup, in a 1-liter Erlenmeyer flask, small amounts of nitric acid were added with slight warming until the initial vigorous reaction was over. The remainder of 150 ml of nitric acid was then added (samples containing little carbon require less nitric acid). The liter flask, with a beaker over the mouth, was heated in a steam autoclave at 175 C for 4 hours. The steam was then replaced by air at an equal pressure so that the sample could be cooled more rapidly. After cooling, the sample was removed and evaporated to dryness. The ash was then ready for analysis.

The sulfite oxidation method of Cooper, Fernstrom, and Miller (1944) was used to measure aeration efficiency.

EXPERIMENTAL RESULTS

The present work was undertaken to find a synthetic medium that would support yeast growth equivalent to that obtained on a natural medium. The synthetic medium finally found to fulfill this requirement is shown in table 1. The highest yield previously reported on a synthetic medium (without the use of large inocula to provide growth factors) is that of Van Lanen (1947). He reported a yield of 11.5 per cent on a modified Williams synthetic medium. This yield was based on the sugar added.

Yields on synthetic medium. In the present investigation *Saccharomyces cerevisiae* y-30 grown on the standard basal medium shown in table 1 gave yields of 42 per cent based on the sugar and asparagine added. This medium contains concentrations of growth factors and minerals equal to twice those found necessary for maximum growth. The amounts necessary for maximum growth were determined on a medium identical with the preliminary test medium shown in table 1 except for the component being varied. The growth curves thus obtained are shown in figure 1*A*, *B*, and *C*.

In the study of the components of the basal medium, no zinc, iron, or copper was added. Later work showed that the unpurified medium contained adequate amounts of all essential minerals.

As indicated in table 1, the glucose and asparagine were not added in excess, since they both function as carbon sources. The asparagine was shown to be utilized by making determinations of the asparagine remaining in the medium at intervals throughout the fermentation. The determinations were made by first hydrolyzing the asparagine with acid to aspartic acid and then determining the aspartic acid by the microbiological assay of Hac and Snell (1945).

The sodium citrate was added to the medium as a buffer. None of the citrate disappeared. This was shown by Dr. Ping Shu, who kindly determined citric acid in both the uninoculated basal medium and the filtrate from the fermented medium and found no difference.

The effect of initial pH is marked. Figure 1*D* shows that the yield obtained by using an initial pH of one unit lower than the pH 5.0 optimum gave a decrease

TABLE 1

Composition of the basal medium

COMPOUND	PRELIMINARY TEST MEDIUM	STANDARD BASAL MEDIUM
	per liter	*per liter*
Glucose*	10 g	10 g
$NH_4H_2PO_4$	6 g	6 g
KH_2PO_4	1 g	0.2 g
$MgSO_4 \cdot 7H_2O$	0.25 g	0.25 g
Sodium citrate	1 g	1 g
L-Asparagine*	1 g	2.5 g
Biotin	40 μg	20 μg
Calcium pantothenate	4 mg	0.5 mg
Inositol	10 mg	10 mg
Thiamine	4 mg	4 mg
Pyridoxine	4 mg	1 mg
Zinc (as sulfate)		400 μg
Iron (as ferrous ammonium sulfate)		150 μg
Copper (as sulfate)		25 μg
Water to 1,000 ml		
pH adjusted to 5.0 with H_3PO_4		

* These compounds serve as carbon sources and are therefore not added in excess.

of 21 per cent in the final yield and an initial pH of one unit above pH 5.0 gave a decrease of 16 per cent in the final yield. The effect of controlling pH throughout the fermentation has not been studied.

Yeast yields vary greatly with glucose concentration. Figure 2*A* shows the increase in yield obtained with decreasing glucose concentration. The 2 and the 4 per cent glucose media were supplied with twice the normal amounts of other constituents of the standard basal medium. The increase in yield at low sugar concentrations is realized in commercial yeast production where the medium is maintained at a low sugar concentration by the continuous addition of a sugar concentrate. It may also be seen from figure 2*A* that the medium containing 2.5 g per liter of asparagine gave highest yields at all sugar levels except 4 per cent. This stimulation by the asparagine was found to increase with increasing sugar concentration. The absolute amount of cell increase brought about by the asparagine may be seen from the following calculations. The basal medium with no asparagine added gave a yield of 23 g per 100 g of glucose. The medium with 1 g asparagine per liter gave a yield of 42.6 g dry yeast per 100 g of glucose. This is a difference of 19.6 g. The total amount of asparagine added to give this

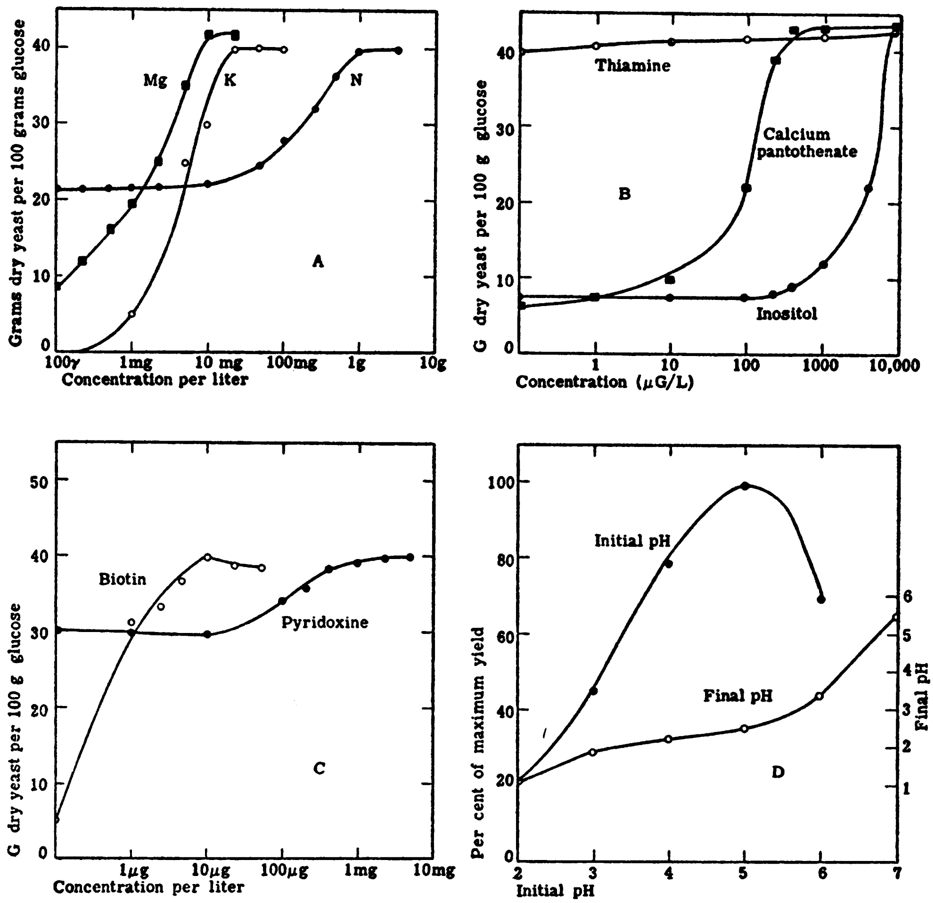

Figure 1. A. Effect of magnesium, potassium, and ammonia nitrogen concentrations on yeast yield. *B.* Effect of thiamine, inositol, and calcium pantothenate concentrations on yeast yield. *C.* Effect of biotin and pyridoxine concentrations on yeast yield. *D.* Effect of initial pH on final yeast yield.

increase was 10 g. Hence, asparagine has a function other than that of a carbon source.

To determine whether or not the yeast required growth factors in addition to the ones added in the basal medium, growth factors as found in natural materials were added as supplements to the basal medium. The results of this experiment are shown in table 2. In no instance was there any improvement over the yield obtained with the basal medium containing 2.5 g asparagine per liter.

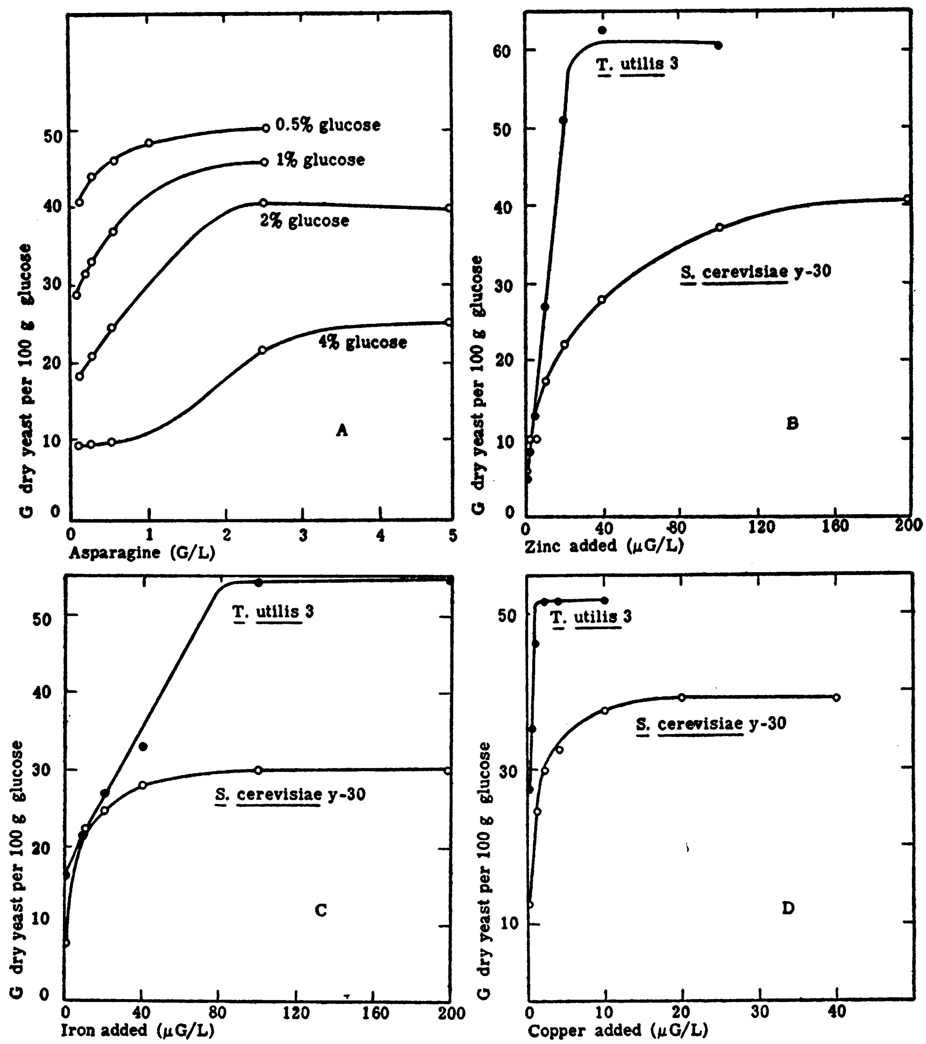

Figure 2. A. Effect of glucose concentration on yeast yield at varying concentrations of asparagine. *B*. Effect of zinc concentration on yeast yield. *C*. Effect of iron concentration on yeast yield. *D*. Effect of copper concentration on yeast yield.

TABLE 2

Effect of natural growth factor supplements on yields

MEDIUM	YIELD (G DRY YEAST PER 100 G GLUCOSE)	
	Run I	Run II
Basal (1 g/L asparagine)	42.6	45
Basal (2.5 g/L asparagine)	50.1	53
Basal (1 g/L asparagine) + (0.5 g/L corn steep liquor)	40.2	42
Basal (1 g/L asparagine) + (ext. of 1 g malt sprouts)	44.3	44.5

Twenty-one different amino acids were tested for their ability to replace asparagine in the basal medium. As shown in table 3, the amino acids were found to fall into three groups. The first group had no effect. This group contained glycine, leucine, glutamic acid, histidine, cystine, tyrosine, and threonine. The L-amino acids were tested at 1.0 and 2.5 g per liter except cystine and tyrosine, which are insoluble at these concentrations. They were used in saturated solution. The DL-amino acids were used at 2.0 and 5.0 g per liter. Group II, consisting of isoleucine, lysine, arginine, ornithine, methionine, tryptophan, and proline, gave slight increases in yield in at least one concentration. The group III amino acids, aspartic acid, alanine, valine, serine, and phenylalanine, showed marked stimulation.

TABLE 3

Ability of single amino acids to replace asparagine

AMINO ACIDS WITH NO EFFECT (YIELD 23–27 G DRY YEAST PER 100 G GLUCOSE)	AMINO ACIDS WITH SLIGHT STIMULATION (YIELD 29–30 G DRY YEAST PER 100 G GLUCOSE)	AMINO ACIDS WITH STIMULATING EFFECT (YIELD 34–53 G DRY YEAST PER 100 G GLUCOSE)
None	DL-Isoleucine	DL-Aspartic acid
L-Leucine	DL-Lysine	DL-Alanine
DL-Glutamic acid	L-Arginine	DL-Valine
L-Histidine	L-Ornithine	DL-Serine
L-Cystine	DL-Methionine	L-Serine
L-Tyrosine	DL-Tryptophane	DL-Phenylalanine
DL-Threonine	L-Proline	
Glycine		

TABLE 4

Effect of serine and alanine on yeast yields in the absence of asparagine

SUBSTANCE ADDED	YIELD (G YEAST PER 100 G GLUCOSE)	FINAL pH
None	23	3.0
L-Asparagine, 1 g per L	42.6	3.1
L-Asparagine, 2.5 g per L	50.1	4.8
DL-Alanine, 2 g per L	39.1	3.35
DL-Alanine, 5 g per L	50.2	3.1
DL-Serine, 2 g per L	48.1	3.1
DL-Serine, 5 g per L	53.2	3.5

It may be seen from table 4 that serine and alanine gave yields equal to those obtained with asparagine. All three gave yields above 50 g of dry yeast from 100 g of glucose. All the glucose added was utilized in these fermentations. Since the pH dropped only from 5.0 to 4.8 in the medium containing 2.5 g asparagine per liter, it might be considered that part of the stimulation by asparagine is due to its ability to maintain the pH constant throughout the fermentation. However, this does not seem to be the case since, with both alanine and serine, yields above 50 per cent were obtained, but the pH dropped from 5.0 to 3.4 and 3.5, respectively.

Table 5 shows the results of an experiment designed to determine whether the stimulation due to alanine and serine was of the same nature as that due to asparagine. In this experiment alanine and serine were added separately and together to a basal medium containing 2.5 g asparagine per liter. In no instance was there any increase in yield over the control. The same yield as the control was obtained even when both alanine and serine were added to the basal medium with 2.5 g asparagine per liter. From this it was concluded that asparagine, alanine, and serine function in a similar way and can be used interchangeably to satisfy the requirement of *Saccharomyces cerevisiae* y-30.

TABLE 5

Effect of serine and alanine on yeast yields in the presence of excess asparagine

SUBSTANCE ADDED TO BASAL MEDIUM (2.5 G/L ASPARAGINE)	YIELD (G YEAST PER 100 G GLUCOSE)	FINAL pH
None	47.0	5.4
DL-Alanine, 2 g per L	47.2	5.7
DL-Alanine, 2 g per L, and DL-Serine, 2 g per L	48.2	5.8
DL-Serine, 2 g per L	47.7	5.7

TABLE 6

Concentration of Zn, Fe, and Cu required for maximum yield

ORGANISM	CONC. REQUIRED FOR MAX. GROWTH		
	Fe	Zn	Cu
	μg/L	*μg/L*	*μg/L*
S. cerevisiae y-30	70	200	12–15
S. cerevisiae 49	150	150	*
Mycotorula lipolytica p-13	25	70	*
T. utilis 3	100	25	3

* Not determined.

Trace metal requirements. Several mineral elements have been found to be essential for the growth of yeast. The amount of each element required is so small that great difficulty was encountered in removing the elements from the medium before growth responses could be determined. In addition to magnesium, potassium, and phosphorus, which are required in relatively large amounts, zinc, iron, and copper have been found to be required in the range of 3 to 200 micrograms per liter.

It will be seen from figure 2*B*, *C*, and *D* that, in the case of *Saccharomyces cervisiae* y-30, cell yield was a logarithmic function of metal concentration, but with *Torulopsis utilis* 3 the relation was linear. Further experiments have shown that *Saccharomyces cerevisiae* 49 also gave a logarithmic response, whereas *Mycotorula lipolytica* p-13 gave a linear response.

Table 6 indicates the amounts of the trace elements required by four different organisms. It may be seen that even organisms within the same species do not have identical quantitative requirements, whereas organisms of different genera have very different quantitative requirements.

The basal media were freed from each element to be tested by the procedures mentioned previously. After purification from iron the medium contained 0.6 micrograms of iron per liter of medium. The purified zinc basal medium contained 1.5 micrograms of zinc per liter, and the copper basal medium contained 0.8 micrograms of copper per liter.

A series of other elements was tested for yeast growth stimulation, but none of the elements showed any effect at the concentrations tested.

Thallium was found to have no effect on yeast growth when added to a medium that had previously been treated to remove Tl^+. The method of purification used was a dithizone extraction procedure done at pH 9.5. Several concentrations of thallium were tried without success. Among the concentrations tested was the one reported by Richards (1932) to be optimum for his yeast.

Added calcium has not shown any effect on yeast yields. The effect of added calcium was tested on a purified medium. The medium without magnesium sulfate was extracted at pH 9.8 with eight 0.1-volume portions of 0.1 per cent 8-hydroxyquinoline chloroform solution. The final 8-hour extraction was done on the reciprocating shaker. The quinoline-metal complex and the excess quinoline were removed by eight 0.1-volume extractions with redistilled chloroform. Then part of the ammonia that had been used to adjust the pH to 9.8 was removed under reduced pressure. This decreased the pH to 7.5. The pH was then adjusted to 5.0 with redistilled hydrochloric acid. The magnesium sulfate was fractionally crystallized from approximately 50 per cent alcohol. Five recrystallizations were made. The second of three crops of crystals was taken each time. The purified magnesium sulfate was added to the pH 5.0 medium. The amount of calcium in the purified medium could not be determined, since the methods that were available for determining calcium were not sensitive to the amount of calcium present in 2 liters of the medium.

Manganese also did not have any effect on yeast growth. After purification the medium contained less than 0.5 micrograms of manganese per liter. The procedure used for the removal of the manganese was identical with that used for removing thallium. Manganese did not have any effect even if the magnesium concentration of the medium was decreased to a level that gave only one-half maximum growth.

Added boron, cobalt, iodine, and tin were also found to have no effect on the yield of yeast. These elements were tested in the unpurified basal medium.

Further experiments indicate that at least one additional element is required for maximum yeast growth. It was found that the ash from 1 liter of purified medium, which contained 0.6 micrograms of iron, when added to the purified medium gave greater yeast growth than could be accounted for on the basis of its iron content. Furthermore, as may be seen from figure 2*B*, the purified medium in the presence of excess iron did not give yields equal to those obtained on the unpurified medium.

Effective aeration. In the following discussion, the term "air flow" refers to the rate at which air is supplied to a fermenter, and the term "effective aeration" refers to the rate at which oxygen dissolves in the fermenter liquid, as measured by the sulfite oxidation method.

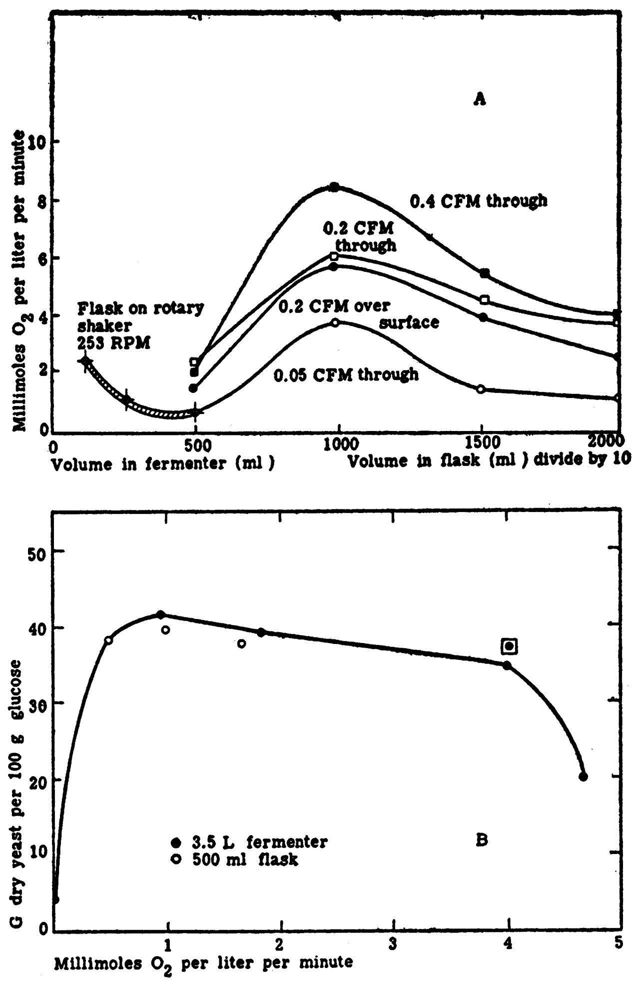

Figure 3. A. Effective aeration measured by sulfite oxidation. *B.* Yeast yields obtained at different effective aeration levels. ⊡ Represents yeast yield corrected for alcohol lost from the fermenter.

Figure 3*A* shows the values of effective aeration found in the 3.5-liter fermenter and in a 500-ml Erlenmeyer flask on a Gump rotary shaker. The points on the curves represent effective aeration values at different liquid volumes at different air flow rates. The fermenter agitator speed was 1,100 rpm. The shaker used for the flask aeration described a 2¼-inch circle 253 times per minute.

It was found that a liquid volume of 25 ml in the 500-ml Erlenmeyer flask gave maximum yields of yeast. From figure 3*A* it is apparent that the effective aeration of the 25-ml volume in the flask is only one-eighth of that attainable in the fermenter.

Figure 3*B* shows the yeast yields obtained at different effective aeration levels. When more than 1 millimole of oxygen is available per liter of medium per minute, the yield of yeast begins to decrease. It can be seen from figure 3*B* that this holds for both flask and fermenter. That this decrease in yield is not due to loss of alcohol from the medium is shown by the point which was corrected for loss of alcohol. This point was obtained by trapping all the alcohol blown out of the fermenter during the fermentation. It was assumed that 100 g of alcohol would yield 70 g of yeast, and the yield was corrected accordingly.

It is important that the yield curve in the flask follow closely the yield curve in the fermenter. This means that if optimal aeration conditions can be achieved in laboratory flask fermentations, optimal yields should be obtained in a large fermenter designed to give the same effective aeration value as that of the flask.

SUMMARY

A synthetic medium has been devised that gave with *Saccharomyces cerevisiae*, strain y-30, 5.33 g of dry yeast per liter of medium. The medium contained 10 g of glucose and 2.5 g of L-asparagine per liter as carbon sources.

Biotin, calcium pantothenate, inositol, pyridoxine, and thiamine supplied the growth factor requirements of the yeast.

Natural materials, such as corn steep liquor and an extract of malt sprouts, when added to the synthetic medium did not increase the yeast yields.

Of 21 amino acids tested, only alanine and serine could completely replace asparagine.

Saccharomyces cerevisiae y-30 was found to require 75 micrograms of iron, 200 micrograms of zinc, and 12 to 15 micrograms of copper per liter of medium. Special procedures for reducing the metal content of the basal medium were found necessary.

The effectiveness of aeration in shake flasks and in a small fermenter was determined by the sulfite oxidation method of Cooper, Fernstrom, and Miller (1944).

A fermenter with high effective aeration is described. With this fermenter it was possible to increase the effective aeration to levels that decreased the yield of yeast. This decrease in yield was shown not to be from loss of alcohol.

REFERENCES

Burkholder, P. R. 1943 Vitamin deficiencies in yeasts. Am. J. Botany, **30**, 206–211.

Burkholder, P. R., McVeigh, I., and Moyer, D. 1944 Studies on some growth factors of yeasts. J. Bact., **48**, 385–391.

Cooper, C. M., Fernstrom, G. A., and Miller, S. A. 1944 Performance of agitated gas-liquid contactors. Ind. Eng. Chem., **36**, 504–509.

de Becze, G., and Liebmann, A. J. 1944 Aeration in the production of compressed yeast. Ind. Eng. Chem., **36**, 882–890.

HAC, LUCILLE R., AND SNELL, E. E. 1945 Microbiological determination of amino acids. III. Assay of aspartic acid with *Leuconostoc mesenteroides*. J. Biol. Chem., **159**, 291–294.

JACKSON, S. H. 1938 Determination of iron in biological materials. Ind. Eng. Chem., Anal. Ed., **10**, 302–304.

JOSLYN, M. A. 1941 The mineral metabolism of yeast. Wallerstein Lab. Commun., **4** (11), 49–65.

LEONIAN, L. H., AND LILLY, V. G. 1942 The effect of vitamins on ten strains of *Saccharomyces cerevisiae*. Am. J. Botany, **29**, 459–464.

RICHARDS, O. W. 1932 The stimulation of yeast growth by thallium, a "bios" impurity of asparagine. J. Biol. Chem., **96**, 405–418.

SANDELL, E. B. 1944 Colorimetric determination of traces of metals. Interscience Publishers, Inc., New York, N. Y.

SHAFFER, P. A., AND SOMOGYI, M. 1933 Copper-iodometric reagents for sugar determination. J. Biol. Chem., **100**, 695–713.

SINGH, K., AGARWAL, P. N., AND PETERSON, W. H. 1948 The influence of aeration and agitation on the yield, protein, and vitamin content of food yeast. Arch. Biochem., **18**, 181–193.

THORNE, R. S. W. 1945 Recent work on the nitrogen nutrition of yeast. J. Inst. Brewing, **51**, 114–126.

VAN LANEN, J. M. 1947 The absorption of niacin by yeast. Arch. Biochem., **12**, 101–111.

WILLIAMS, R. J. 1941 Growth-promoting nutrilites for yeast. Biol. Rev. Cambridge Phil. Soc., **16**, 49–80.